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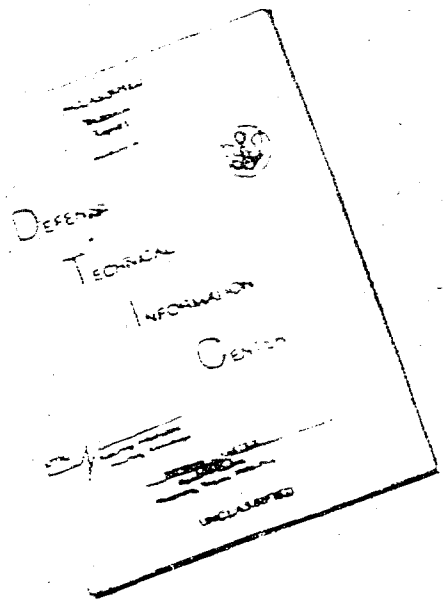

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CULTIVATION OF CHICKEN POX VIRUS IN DEVELOPING CHICK EMBRYOS

[Following is the translation of an article by [illegible] Department of Epidemiology and Medical Parasitology, Kharkov Institute for the Advanced Training of Doctors, published in the Russian-language periodical Voprosy Virusologii (Problems of Virology), No 10, 1965, pages 803-804. It was submitted on 4 Jul 1964.]

During recent years both abroad and in our country investigations have been carried out on the cultivation of the varicella virus and a study of its properties.

In 1952 Weller and Stodart described eosinophilic intranuclear inclusions in a tissue culture of a human embryo which had been infected with the contents of varicella vesicles. Arakava, Kondo, and Sonz (1955) attempted to isolate the chicken pox virus by the method of intracerebral infection of white mice. Taylor and Robinson (1956) undertook the investigation on the cultivation of the chicken pox virus from vesicle contents in tissue cultures of human fibroblasts and on amnion cells.

Togunova and Bronshteyn [17] took on the task of studying the chicken pox virus with passaging through animal organisms, that is, the direct seeding of material in a tissue medium with the subsequent detection of elementary bodies.

In the present report we are presenting data on the cultivation of the chicken pox virus on the chorio-allantoic membranes of a chick embryo and the results of the subsequent study of certain properties of the virus.

Materials and Methods

As material for contamination we used the blood from a patient with chicken pox. 10-day old chick embryos were used for cultivation of the virus. Using a lancoolate needle punctures were made on the surface of the shell and with a syringe 0.3 ml of the virus was inoculated into the chorio-allantoic membrane. The openings were sealed with sterile melted paraffin with wax. The injected embryos were incubated in an incubator at a temperature of 37° for 5 days.

The chorio-allantoic membrane of the autopsied embryos was checked for bacterial contamination. The extracted chorio-allantoic membranes were crushed in a mortar with sterile crushed glass and a suspension was prepared in physiological solution with the addition of antibiotics on the basis of 500 AU of penicillin and 500 AU of

streptomycin in 1 ml. The suspension was prepared in a dilution of 1:4. The resulting suspension was centrifuged for 10 min at 2,000 rpm. The supernatant fluid was used for the passages.

For the isolation of chicken pox virus in chick embryos we used 30 specimens of blood taken from 30 patients. All told 20 strains were isolated which had undergone 0 to 15 passages and 5 strains which had undergone 40-50 passages. The blood was taken from children in the ages from 3 months to 8 years who had a severe form of chicken pox and copious exudation. In 4 cases the blood was taken from children aged 10-13 and in one case from a 25 year old adult who had come from Cuba.

Results of the Investigation

For studying the accumulation of chicken pox virus on the embryonic membrane of a developing chick embryo 0.2 ml of the virus-containing material was inoculated in a dilution of 1:4. Macroscopic changes were studied after 24, 48, 72, 96, and 120 hours. 24 hours after infection of the embryos visible changes were not observed on the chorio-allantoic membrane; in 48 hours microscopic changes were noted on the embryonic membrane and these were especially distinct after 72-96 hours.

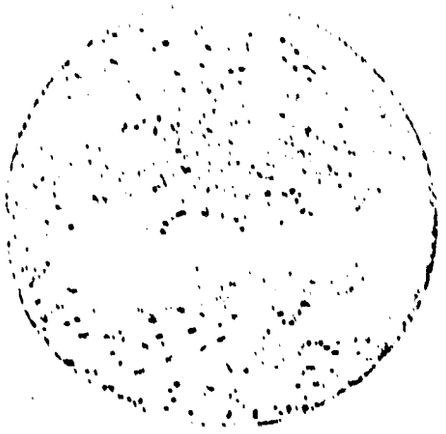
Macroscopic changes on the embryonic membrane were in the form of small nodular formations with the size of a pinhead around which there were weakly noticeable white-colored zones in the form of a corona with a radius of 0.5-0.6 cm. The intensity of the white zone decreases with removal from the nodule.

During repeated passages (3-5 passages) on embryonic membrane around the nodular formations there are single punctulate dull-white formations in the thickness of the membrane. These we conditionally named daughter foci of affection. The number of these foci was not always the same. In the majority of cases 3-4 foci were observed and sometimes the number of these foci reaches 8-10. The described changes developed in an invariable embryonic membrane. Only a certain anemia of the membrane was noted and there was no edema and hemorrhagic changes.

Chorio-allantoic membranes with clearly expressed macroscopic changes after 96 hours following inoculation of the virus were fixed in Dyubosk-Brazil-Buens mixture, thin paraffin media [illegible] were prepared, and they were stained with hematoxylin-eosin, triple Unna stain, and by the Romanov-Giemsa method.

Microscopic changes in the chorio-allantoic membrane were characterized by the appearance of focal lesions in the ectodermal epithelium. The ectoderm was thickened as a result of hyperplasia

and hypertrophy of epithelial cells. In places in the ectodermal layer large gigantic cells were revealed which were oval in shape and had a large number of nucleoi - from 6 to 8-12. Apparently the appearance of multinuclear cells is the result of the amitotic division of nucleoi during the interaction of the virus with the cell. Part of the nucleoi in gigantic cells are in a state of degeneration, intranuclear inclusions are minute (see drawing).



Chorio-allantoic membrane affected by virus. Minute-punctate focal changes. Staining with hematoxylin-eosin. X100.

Titration of the virus, which was obtained from the embryos in various periods after incubation, showed that maximum titers were noted 72 hours after inoculation of the virus. In the first passages (3-4) small titers (10^{-6}) were noted, but with subsequent passages the titers reached a level which exceeded the initial titer by 2-4 dilutions. Thus, if on the 15th passage of the virus the titer equaled 10^{-8}, then by the 20-30-40th passages it reached 10^{-10}.

During the study of the homagglutinating activity of the chicken pox virus it was established first that the embryonic membrane strains of ovovaricella which we isolated possessed a homagglutinating activity. Agglutinated particularly well were the erythrocytes of sheep, human blood group O, and chick embryos. Homagglutinating activity of the virus was detected in low titers and this property was not always permanent. The results of the investigation regarding the determination of the homagglutinating activity of the virus will be presented in a separate report.

Conclusions

1. The virus of chicken pox adapts readily and multiplies

in the chorio-allantoic membranes of a chick embryo.

2. A virus which has undergone several passages on chorio-allantoic membrane causes macroscopic changes in it.

3. The chicken pox virus possesses a hemagglutinating capacity.

Literature

1. Togunova, A. I., Bronshteyn, N. O., Zh. mikrobiol., 1938, Vol 20, No. 3-4, p 42.